Phylogeography and population genetics of social parasitism in *Myrmica* ants

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ACADEMIC DISSERTATION

To be presented for public examination
with the permission of the Faculty of Biological and Environmental Sciences of
the University of Helsinki in Auditorium 2041 in Biocenter 2 (Viikinkaari 5)
on November 30th 2012 at 9 a.m.

HELSINKI 2012

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ISBN 978-952-10-8419-5 (paperback)
ISBN 978-952-10-8420-1 (PDF)
http://ethesis.helsinki.fi

Unigrafia
Helsinki 2012

Tänään

Tänään on juuri oikea aika
muurahaisen lentää kuuhun
ja tehdä siellä semmoinen taika
että namusia sataa suuhun.

– Kirsi Kunnas, Tiitiäisen satupuu

Contents

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Leppänen, J., Vepsäläinen, K. & Savolainen, R. 2011. Phylogeography of the ant *Myrmica rubra* and its inquiline social parasite. *Ecology and Evolution* 1: 46-62.

II Leppänen, J., Vepsäläinen, K., Anthoni, H. & Savolainen, R. 2012. Comparative phylogeography of the ants *Myrmica ruginodis* and *Myrmica rubra*. *Journal of Biogeography*, in press.

III Leppänen, J., Seppä, P., Vepsäläinen, K. & Savolainen, R. Microsatellite markers reveal substantial genetic differentiation between the ant *Myrmica rubra* and its microgynous social parasite—a sign of incipient sympatric speciation? *Manuscript.*

IV Leppänen, J., Seppä, P., Vepsäläinen, K. & Savolainen, R. Reproductive isolation of the ant *Myrmica rubra* and its microgynous social parasite—mating preferences and genetic origin of males. *Manuscript.*

Table of contributions

	I	**II**	**III**	**IV**
Original idea	RS, KV	RS	RS, KV	RS, KV
Design	RS, JL	JL, RS	JL, RS, PS	JL, RS, PS
Data collection	RS, KV, JL[1]	RS, KV, JL[1]	JL, RS, KV	JL, RS, KV
Laboratory work	JL	HA, JL	JL	JL
Analyses	JL	JL, HA	JL, PS	JL, PS, KV
Manuscript preparation	JL, KV, RS	JL, KV, RS	JL, PS, RS, KV	JL, PS, RS

RS = Riitta Savolainen, KV = Kari Vepsäläinen, JL = Jenni Leppänen, HA = Hannele Anthoni, PS = Perttu Seppä
[1]and several colleagues

ABSTRACT

One of the main questions in evolutionary biology is what causes genetic differentiation among populations and how do new species originate. In this thesis I studied genetic differentiation of populations by testing how changes in the environment and behaviour have affected populations of *Myrmica* ants (Formicidae, Hymenoptera).

First, I used a phylogeographic approach to find out how climatic changes during and after the last glaciation have affected populations of two Palearctic ant species *Myrmica rubra* and *M. ruginodis*. The aim was to locate their glacial refugia and postglacial recolonization routes, and to test whether differences in their cold tolerance and life-history traits have affected their phylogeographic structures and locations of their glacial refugia.

Second, I studied the effect of behavioural differences on populations of *M. rubra*. I examined genetic differentiation and reproductive isolation of an intraspecific microgynous social parasite and its macrogynous host using both genetic and behavioural approaches. The aim was to clarify whether social parasitism as an alternative reproductive tactic may lead to speciation in sympatry.

The results of this thesis showed that differences in life-history traits have had no effect on the phylogeographic structures of *M. rubra* and *M. ruginodis*. Both species have survived the last glaciation in several refugia in Southern Europe but probably also in regions further east and north. Circumstantial evidence suggests that the more cold-tolerant *M. ruginodis* has survived in refugia more north than *M. rubra*. Both species have recolonized the northern areas from the south-west and south-east, and probably also from the east.

Both genetic and behavioural evidence suggested that in *M. rubra* the parasite and host are reproductively isolated. They differed substantially in their nuclear DNA, indicating that the parasite is in the process of speciation. However, the mitochondrial lineages of the host and the parasite have not yet diverged.

This thesis brings new information on the history of Palearctic insects and the effects of the past environmental changes on their populations. This thesis also clarifies the evolution of social parasitism and provides new evidence for a probable example of sympatric speciation through an alternative reproductive tactic, social parasitism.

TIIVISTELMÄ

Yksi evoluutiobiologian tärkeimmistä kysymyksistä on, mikä aiheuttaa populaatioiden geneettistä erilaistumista ja uusien lajien syntymistä. Tutkin väitöskirjatyössäni tekijöitä, jotka vaikuttavat populaatioiden geneettiseen erilaistumiseen ja selvitin, miten elinympäristön ja käyttäytymisen muutokset ovat vaikuttaneet *Myrmica*-suvun muurahaisten (Formicidae, Hymenoptera) populaatioihin.

Ensiksi tutkin fylogeografisten menetelmien avulla elinympäristön muutosten vaikutusta kahden Palearktisen muurahaislajin, siloviholaisen (*Myrmica rubra*) ja ryppyviholaisen (*M. ruginodis*), populaatioihin. Tavoitteenani oli paikallistaa lajien jääkauden aikaiset refugiot (turvapaikat) ja jääkauden jälkeiset leviämisreitit, sekä selvittää, miten erot lajien kylmänkestävyydessä ja elinkierrossa ovat vaikuttaneet niiden fylogeografiseen rakenteeseen ja refugioiden sijaintiin.

Toiseksi, tutkin käyttäytymiserojen vaikutuksia siloviholaispopulaatioihin. Selvitin lajinsisäisen sosiaalisen mikrogyyniloisen ja sen makrogyyniemännän geneettisen erilaistumisen määrää ja lisääntymisisolaatiota tutkimalla sekä niiden genetiikkaa että käyttäytymistä. Tavoitteenani oli selvittää, voiko sosiaalinen loisinta vaihtoehtoisena lisääntymistaktiikkana johtaa lajiutumiseen.

Erot lajien elinkierrossa eivät ole vaikuttaneet niiden fylogeografiseen rakenteeseen. Molemmat lajit ovat selviytyneet viimeisen jääkauden aikana useassa refugiossa Etelä-Euroopassa, mutta myös itäisemmillä ja pohjoisemmilla alueilla. Kylmänkestävämpi ryppyviholainen on luultavasti selviytynyt pohjoisemmissa refugioissa kuin siloviholainen. Lajit ovat todennäköisesti levinneet jääkauden jälkeen pohjoiseen lounaasta, kaakosta sekä luultavasti myös idästä.

Sekä genetiikkaan että käyttäytymiseen perustuvat tulokset viittaavat siihen, että loisen ja sen emännän välillä on lisääntymisisolaatio. Niiden tuman DNA poikkesi merkitsevästi toisistaan ja loinen on luultavasti lajiutumassa emännästään. Loisen ja emännän mitokondrion DNA:n linjat eivät kuitenkaan ole vielä eriytyneet toisistaan.

Väitöskirjatyöni tuo uutta tietoa Palearktisten hyönteisten historiasta ja siitä, miten elinympäristön muutokset ovat vaikuttaneet niiden populaatioihin viimeisen jääkauden aikana ja sen jälkeen. Työni valaisee sosiaalisen loisinnan evoluutiota ja antaa uusia viitteitä sympatrisesta lajiutumisesta tilanteessa, jossa lajiutumisen syynä on vaihtoehtoinen lisääntymistaktiikka, sosiaalinen loisinta.

SUMMARY

1. INTRODUCTION

The most important basis of evolution is genetic variation of individuals (Dobzhansky, 1937; Ridley, 1996). Within and among individuals, different alleles are present, which enables evolutionary mechanisms such as genetic drift and selection to act and influence which set of genes will be passed to the next generation (Fig. 1). Owing to genetic variation populations can become genetically differentiated (Hedrick, 2000), which may eventually lead to speciation (Dobzhansky, 1937, Fig. 1).

Genetic differentiation of populations is prevented by gene flow, and therefore, genetic differentiation is usually smaller within than among populations, and among geographically close than distant populations (Wright, 1942; Hedrick, 2000). One of the main interests in evolutionary biology is to study what prevents gene flow among populations and causes their genetic differentiation. Underlying reasons can be many, such as environmental factors and behavioural differences (Fig. 1). To have a comprehensive view of what has driven populations genetically apart, their genetic variation and differentiation should be studied both on different geographic and evolutionary time scales. An informative approach to study the mechanisms behind genetic differentiation of populations is to combine history, geography, ecology and genetics, and to use a phylogeographic approach (Avise, 2000).

1.1. Phylogeography—populations in space and time

Phylogeography is the study of the geographic distribution of genetic lineages, especially within species and among closely related species (for explanation of genetic lineage, see 3.4.). Phylogeography helps us to understand the historical processes that have shaped the genetic constitution of species and how past environmental changes have affected genetic variation and differentiation of populations. One important aspect of phylogeography is the effects of the Pleistocene climatic cycles on populations (Avise, 2000).

The genetic constitution of temperate species has been affected by the demographic processes during the Pleistocene climatic cycles (Avise, 2000; Hewitt, 2004). In the Pleistocene, repeated glacial and interglacial periods altered the distribution and abundance of populations. During the Last Glacial Maximum (LGM), a large part of Eurasia was covered by ice (Svendsen *et al.*, 2004) and populations of temperate species decreased and retreated into restricted areas referred to as glacial refugia (Hewitt, 1996; Stewart *et al.*, 2010). Populations living in different refugia became isolated from each other, for example, owing to unsuitable habitat, which prevented gene flow among them. Eventually new genetic lineages formed and refugial populations became genetically differentiated from

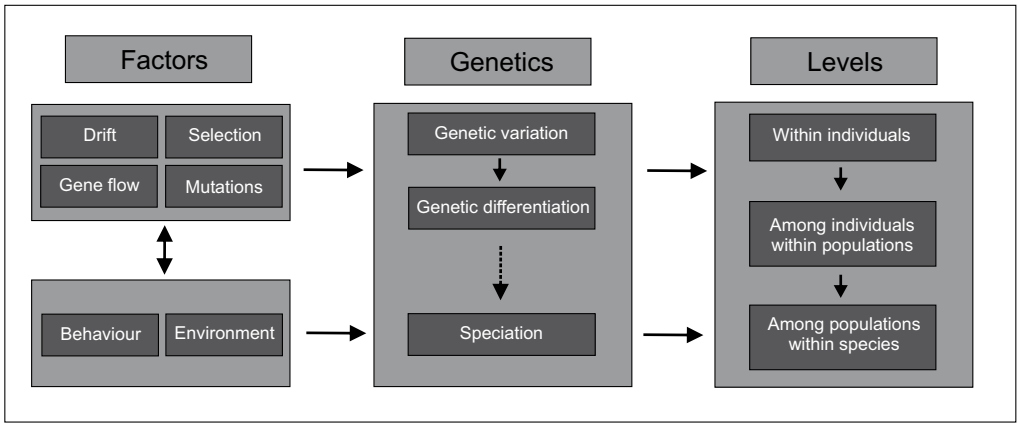


Fig. 1. A diagram showing the origin and levels of genetic differentiation.

each other. After the ice sheet started to retreat and the interglacial period began, many populations grew and spread out from the refugia. They recolonized areas previously covered by ice, sometimes coming into secondary contact with other refugial populations and their genetic lineages (Taberlet *et al.*, 1998; Hewitt, 1999). All these events have left imprints on the distribution and genetic constitution of populations and today most temperate species are phylogeographically structured, displaying regionally restricted genetic lineages (Avise, 2009).

Comparative phylogeography is the study of geographic distributions of genetic lineages among species (Bermingham & Avise, 1986). If the genetic lineages of species have congruent distribution, they species share a phylogeographic structure. In the northern hemisphere, for example, the shared phylogeographic structures have been used to deduce the major locations of Pleistocene refugia and subsequent postglacial recolonization routes (Bermingham & Avise, 1986; Taberlet *et al.*, 1998; Hewitt, 1999; Schmitt, 2007). On the other hand, if the genetic lineages of two species are not congruent and the species do not share a phylogeographic structure, causes for the underlying disparities render interesting prospects for a comparative study (Avise, 1998). Incongruent phylogeographic structures may result, for example, from species-specific differences in habitat and dispersal (Zink, 1996; Avise, 1998; Papadopoulou *et al.*, 2008, 2009). These factors may be difficult to differentiate, however, from distributional and historical differences among species. Therefore ideal objects of a comparative phylogeographic analysis are closely related species that have sympatric distributions (Pauls *et al.*, 2009).

1.2. Modes of speciation

One of the most difficult tasks in evolutionary biology is to define a species (Templeton, 1989). Several different species concepts have been proposed, the most widely used being the biological species concept (Mayr, 1942), which defines species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". The other concepts define species, for example, based on morphology, ecology or phylogenetics (Templeton, 1989). None of the species concepts apply, however, for all taxa in all circumstances and often species boundaries are difficult to draw (Templeton, 1989). Therefore, rather than concentrating on defining species, a more interesting topic in evolution is to study the process of speciation—why and how new species arise?

Isolating mechanisms, which prevent gene flow among populations, are a prerequisite for speciation. These isolating mechanisms can be either extrinsic or intrinsic and their distinction is a key factor in defining the modes of speciation (Fitzpatrick *et al.*, 2009). Traditionally, speciation has been divided into three different modes according to the geographic scale: allopatric, parapatric and sympatric speciation (Mayr, 1942; Bush, 1975).

In allopatric speciation, populations diverge in geographic isolation and there is no gene flow among them due to extrinsic barriers such as mountains, rivers or unsuitable habitat. Many factors such as genetic drift, bottlenecks and adaptation to local environment may drive the isolated populations genetically apart (Mayr, 1942; Bush, 1975).

In parapatric speciation, ranges of populations are situated next to each other and they meet only in narrow contact zones. In these contact zones, there is little gene flow among the neighbouring populations that are mostly isolated by extrinsic, geographic barriers as in allopatric speciation. Eventually, populations become genetically differentiated from each other owing to the effect of isolation by distance and local adaptation (Mayr, 1942; Bush, 1975). Representative examples of parapatric speciation are the ring species, such as salamanders in the *Ensatina* complex (Wake, 1997).

In the most extreme mode of speciation, sympatric speciation, populations differentiate from each other in the same area, without geographic barriers (Mayr, 1942; Bush, 1975). Thus, in sympatric speciation intrinsic barriers must develop to prevent populations from interbreeding (Mayr, 1942; Bush, 1975; Via, 2001; Fitzpatrick *et al.*, 2009). One of the most important factors in sympatric speciation is disruptive selection and positive assortative mating, the tendency to mate with phenotypically similar individuals. Assortative mating can lead to reproductive isolation and prevent gene flow among populations (Smith, 1966; Gavrilets, 2003).

Allopatric speciation has traditionally been treated as a null hypothesis for speciation (Coyne & Orr, 2000; Fitzpatrick *et al.*, 2009), although recently an increasing number of studies have focussed on the other modes of speciation, especially on sympatric speciation (Turelli *et al.*, 2001; Via, 2001; Fitzpatrick *et al.*, 2009). Sympatric speciation is a highly debated topic since

it is often difficult or even impossible to prove that populations have not diverged in allopatry (Coyne & Orr, 2000; Fitzpatrick *et al.*, 2009). Sympatric speciation is possible in theory (Gavrilets, 2003; Thibert-Plante & Hendry, 2011) and it has been suggested to occur in several taxa (Via, 2001; Fitzpatrick *et al.*, 2009), but so far no examples exist where allopatry can confidently be ruled out (Coyne & Orr, 2000; Fitzpatrick *et al.*, 2008, 2009). Most of the examples of sympatric speciation draw criticism because they rarely fulfil all the criteria Coyne & Orr (2000) have defined for sympatric speciation (reviewed by Fitzpatrick *et al.*, 2009): the taxa are sympatric sister groups that are in reproductive isolation and have likely never been in allopatry.

The traits mediating sympatric speciation are usually ecological. Competition for different resources can lead, for example, to a shift in host species or diet (Via, 2001). One of the best known examples are the cichlid and stickleback fishes that are specialised in different diets and occupy separate habitats within a lake (McKinnon & Rundle, 2002; Barluenga *et al.*, 2006). The sympatric morphs of these fishes differ, for example, in their size or colouration, and they prefer to mate with individuals of the same morph as themselves. Among invertebrates, probably the most famous examples are the host races of herbivorous insects (Bush, 1969; Feder *et al.*, 1988, but see also 2003; Frantz *et al.*, 2006). Larvae of the apple maggot flies (*Rhagoletis pomonella*) are host-specific fruit parasites that use hawthorn (*Crataegus* spp.) as their host. When the apple tree (*Malus pumila*) was introduced to North America in the 18th century, some individuals shifted to use this more abundant host. A new apple race of *Rhagoletis* formed when flies began to mate with individuals using the same host as themselves. Nowadays the apple and hawthorn races differ genetically and little gene flow occurs between the races (Olsson *et al.*, 2006). Another promising example of sympatric speciation in insects are the obligate social parasites of ants (e.g., Wasmann, 1909; Elmes, 1978; Buschinger, 1990; Bourke & Franks, 1991; Mayr, 1993).

1.3. Evolution of social parasitism

A social parasite takes advantage of interactions and co-operation between social organisms (Buschinger, 1986). Social parasitism is common in social insects of the order Hymenoptera, such as bees and ants, where the social parasite may be either a social insect or another invertebrate species (Hölldobler & Wilson, 1990). Here I will concentrate on the former and especially on ants.

In ants, non-fertilised haploid eggs develop into males that live only for a short period in the mating season, whereas fertilised diploid eggs develop into more long-lived females, either queens or workers. Caste determination of females may be affected by several factors, such as nutrition, environment and genetics (Smith *et al.*, 2008) as well as by behaviour of queens and workers (Brian, 1954, 1970; Brian & Carr, 1960; Elmes & Wardlaw, 1983; Keller, 1995). The queens mate and are able to produce both haploid males and diploid females whereas workers have diminished reproductive organs, they do not mate, and can thus produce only haploid males (Brian, 1953, 1969; Elmes, 1991b). However, when the queen is present in the nest, workers usually only lay trophic eggs (Bourke, 1988; Walin *et al.*, 1998; but see Brian, 1969 and Smeeton, 1981). The workers take care of all the colony tasks, and nurture and feed the queen and its offspring (Wilson, 1971), which makes an ant colony a tempting target for social parasitism.

Social parasitism in ants may occur both within (intraspecific) or between species (interspecific). A social parasite uses the colony of its host to enhance its own reproduction and fitness. The parasite may be host-specific or use several species as its hosts, and it may be either entirely dependent on its host colony (an obligate social parasite) or parasitize the host only occasionally (a facultative social parasite). Four types of social parasitism have been described: guest relations, temporary parasitism, slavery (dulosis) and the most advanced type—inquilinism (Buschinger, 1986).

In inquilinism, the parasite, referred to as an inquiline, coexists with the host queen without killing it (Buschinger, 1986). Sometimes social parasites that kill the queen are also referred to as inquilines (Wcislo, 1987; Buschinger, 1990), but, here I will use the term only for those parasites that coexist with their host queens. Inquiline parasites share many behavioural traits, one of them being the reduction of the worker force (Wilson, 1971). When the parasite has intruded the nest of the host, it produces only sexual offspring, gynes (potential future queens) and males, while the host queen produces only workers (Buschinger, 1986). The reduction of worker force is beneficial for the inquiline, since workers rarely reproduce (Brian, 1953, 1969; Bourke, 1988; Walin *et al.*, 1998) and they are thus less profitable for the fitness of the inquiline than sexual offspring. The reduction of workers has,

however, also a negative side effect of inquilines being entirely dependent on their hosts. Thus, inquilines are usually local and rare, and their populations are small unlike those of other social parasites that have workers (Buschinger, 1986; Hölldobler & Wilson, 1990).

Another shared trait of inquilines is their morphology (Wilson, 1971). Inquiline ant queens usually share several morphological features such as shiny body surface, large postpetiole and small size. Inquiline ant queens are smaller than their host queens, often the size of the host workers, or even smaller (Bourke & Franks, 1991; Nonacs & Tobin, 1992). Small size, miniaturisation of the queens, may be one of the most important traits in evolution of inquilinism (Wheeler, 1910). Since inquilines do not establish they own colony, they need less body reserves than their hosts and thus miniaturisation of the inquiline can be a consequence of dependent colony founding (Keller & Passera, 1989; Stille, 1996). Another possible explanation is that miniaturisation could be a consequence of escaping the caste control of workers. Inquilines may benefit from their small size, since small larvae need less food to cross the caste threshold and develop into queens than do large ones (Elmes, 1976; Nonacs & Tobin, 1992; Aron *et al.*, 1999) and in the presence of the queen workers may favour small larvae (Brian & Carr, 1960).

The evolution of social parasites has intrigued evolutionary biologists over a century (Buschinger, 2009). Many obligate social parasites are closely related to their hosts (e.g. Wilson, 1971; Buschinger, 1986, 1990; Bourke & Franks, 1991; Heinze, 1995; Savolainen & Vepsäläinen, 2003; Sumner *et al.*, 2004; Smith *et al.*, 2007; Feitosa *et al.*, 2008; Huang & Dornhaus, 2008; Jansen *et al.*, 2010), which is called Emery's rule (Emery, 1909; Le Masne, 1956). The rule has been divided into a loose and strict form (Ward, 1989). In the strict form, the parasite and host are sister taxa, thus most closely related to each other than to any other taxon, whereas in the loose form the parasite and host are not sister taxa but only closely related. Emery's rule applies to many social parasites and their hosts (Wilson, 1971; Buschinger, 1986, 1990; Bourke & Franks, 1991; Huang & Dornhaus, 2008), especially to inquilines (Huang & Dornhaus, 2008). In most studies, however, only the loose form has been applied (Agosti, 1994; Ronquist, 1994; Heinze, 1995; Pedersen, 1996; Ward, 1996; Sanetra & Buschinger, 2000; Parker & Rissing, 2002; Carpenter & Perera, 2006), whereas the strict rule has only been applied to selected groups, such as allodapine bees (Smith *et al.*, 2007) and some ants in the genera *Leptothorax* (Heinze, 1995), *Acromyrmex* (Sumner *et al.*, 2004), *Ectatomma* (Feitosa *et al.*, 2008) and *Myrmica* (Savolainen & Vepsäläinen, 2003; Jansen *et al.*, 2010).

The close relationship of social parasites and their hosts may imply that species of obligate social parasites have evolved from their hosts sympatrically through intraspecific social parasitism (Wasmann, 1909; Elmes, 1978; Buschinger, 1990; Bourke & Franks, 1991). Furthermore, social parasitism may have evolved in queen dimorphic ants as an alternative reproductive tactic. According to one hypothesis, small queens have become social parasites of the large ones, followed by reproductive isolation and genetic differentiation of the parasites from their intraspecific hosts (Elmes, 1978; Pearson & Child, 1980; Pearson, 1981; Buschinger, 1990; Bourke & Franks, 1991).

Most studies have investigated Emery's rule and the evolution of social parasites and their speciation by examining phylogenetic relationships of species (Huang & Dornhaus, 2008). This is problematic since many processes may have taken place since speciation. For example, some of the taxa may have become extinct or be missing from the phylogeny owing to too scarce sampling, or parasite may have switched its host (Bourke & Franks, 1991; Smith *et al.*, 2007; Jansen *et al.*, 2010). In addition, when speciation has already occurred, it is difficult to distinguish whether speciation has occurred in sympatry or allopatry followed by sympatry (Smith *et al.*, 2007). For these reasons, wrong conclusions on the history of species can easily be drawn. Therefore, it would be ideal to study speciation of social parasites where the speciation process has not yet been completed, but where it is still ongoing. Species with intraspecific social parasitism would be ideal objects to study the speciation of social parasites.

1.4. The ants *Myrmica rubra* and *M. ruginodis*

The ant genus *Myrmica* consists of over 200 species of which most occur in the Holarctic region (Seifert, 1988; Radchenko, 1995; Radchenko & Elmes, 2003, 2010; Bolton *et al.*, 2006; Czechowski *et al.*, 2012). *Myrmica* species have an annual life-cycle with two different brood types—rapid and slow—and an obligatory winter diapause (Brian, 1951, 1954, 1956; Elmes, 1991b; Kipyatkov, 1993; Kipyatkov & Lopatina, 1997; Kipyatkov *et al.*, 2005). The rapid brood develops

into adults in a single growing season, whereas the slow brood overwinters and ends its metamorphosis during the following summer. The rapid brood consists of workers whereas the slow brood includes all castes (Elmes, 1991b). In *Myrmica* polygyny (multiple-queen nests), polycalism (multiple-nest colonies) and colony fission are common (Elmes, 1991b; Elmes & Keller, 1993), which makes their colonies vulnerable to social parasitism. Consequently, *Myrmica* is especially rich in social parasites: of the circa 200 species, 14 are socially parasitic, including both temporary and obligate social parasites (Bolton, 1988; Radchenko & Elmes, 2003; Bolton *et al*., 2006; Jansen *et al*., 2010).

Myrmica rubra (Linnaeus, 1758) and *M. ruginodis* Nylander, 1846 are closely related (Jansen *et al*., 2010) and common Palearctic species (Czechowski *et al*., 2012). *Myrmica ruginodis* is a more cold-tolerant species than *M. rubra* (Maysov & Kipyatkov, 2011) and it is distributed within continuous area of the present permafrost, whereas the distribution of *M. rubra* scarcely reaches the southern limit of the continuous permafrost (Romanovsky *et al*., 2007; Czechowski *et al*., 2012).

Species differ in their life-history characteristics. The colonies of *M. ruginodis* are more often monogynous (single-queen nests) and monocalic (single-nest colonies), whereas colonies of *M. rubra* are typically polygynous (multiple-queen nests) and polycalic (multiple-nest colonies). The life-history of *M. ruginodis* is also characterised by more efficient dispersal than that of *M. rubra* (Elmes, 1980; Seppä & Pamilo, 1995).

Since *M. rubra* and *M. ruginodis* are closely related (Jansen *et al*., 2010) and largely sympatric (Czechowski *et al*., 2012), they are ideal species for a comparative phylogeographic study (Zink, 1996; Pauls *et al*., 2009). As the species have extensive Palearctic distributions and they are relatively cold-tolerant (Kipyatkov, 2001; Czechowski *et al*., 2012), they may have survived in many Pleistocene refugia and are thus optimal species to study phylogeoraphic patterns of western Palearctic species. *Myrmica rubra* and *M. ruginodis* also enable the investigation of how differences in cold tolerance and life history tactics, such as dispersal (Elmes, 1980; Seppä & Pamilo, 1995), may affect the populations and phylogeographic sructures of species.

Another difference between *M. rubra* and *M. ruginodis* is the behaviour of queens. Both species have two size classes of queens, large macrogynes and small microgynes (Brian & Brian, 1949, 1955; Elmes, 1973). In *M. ruginodis*, the queen morphs overlap whereas those of *M. rubra* hardly overlap although intermediate forms sometimes exist (Brian & Brian, 1949, 1955; Elmes, 1973, 1976). The size differences of males are not so pronounced as in the queens (Elmes, 1991a; Seifert, 1993). Microgynes of *M. ruginodis* behave as normal queens, produce workers and are able to establish their own nests (Weir, 1959; Elmes, 1991a). Brian & Brian (1955) treated *M. ruginodis* queen morphs as subspecies, but Seppä (1992, 1994) found no genetic clusters indicating genetic differentiation between the two queen morphs. Unlike microgynes of *M. ruginodis*, those of *M. rubra* are inquilines, obligate social parasites of the macrogynes (Elmes, 1978; Pearson & Child, 1980; Pearson, 1981). As the queen morphs of *M. rubra* produce only morphs of their own kind, queen size dimorphism is presumably genetically regulated (Elmes, 1976).

Pearson & Child (1980) and Pearson (1981) strongly suspected that the microgynous morph of *M. rubra* is a separate species and Elmes & Keller (1993) referred to it as *M. rubra microgyna*. Later, based on the size difference between the morphs, Seifert (1993) described the microgynous morph as a separate species, *Myrmica microrubra*. However, 13 years later Steiner *et al.* (2006) used genetic data to synonymize it with *M. rubra* and concluded that microgynes were not social parasites, thus discarding the parasitic features of the microgynes: dependency on its host (Elmes, 1976, 1991b) and excessive production of sexual offspring (Pearson, 1981).

The subsequent studies have justified parasitic status of the microgyne (Seifert, 2007, 2010; Vepsäläinen *et al*., 2009) but supported the synonymization (Vepsäläinen *et al*., 2009). Some genetic differentiation has been found, however, between the queen morphs (Steiner *et al*., 2006; Vepsäläinen *et al*., 2009) and several authors have suggested that the social parasite of *M. rubra* may be undergoing speciation and represent an example of sympatric speciation through intraspecific social parasitism (Savolainen & Vepsäläinen, 2003; Seifert, 2007, 2010; Vepsäläinen *et al*., 2009). Thus far, the extent of genetic differentiation between *M. rubra* and its parasite and their reproductive isolation has not been documented.

Since *M. rubra* is common and widely distributed (Czechowski *et al*., 2012), and the parasite is often found wherever dense *M. rubra* populations are present (Elmes, 1976), *M. rubra* and its parasite provide a good system to study the modes and processes of speciation of social parasites.

2. OUTLINE OF THE THESIS

In this thesis I studied factors behind genetic differentiation of populations of *Myrmica* ants in different evolutionary and geographic scales. I investigated the populations both in historical scale using phylogenetics and in more short-time scale with population genetics, both globally and locally.

First, I studied phylogeography of closely related and largely sympatric species *M. rubra* and *M. ruginodis* in the Palearctic region (Chapters **I** and **II**). The aim was to localise their glacial refugia and subsequent postglacial recolonization routes, and to test whether the differences in cold tolerance and life-history traits have had an effect on their phylogeographic structure, population differentiation and locations of their refugia.

Second, I examined genetic differentiation of *M. rubra* and its microgynous social parasite on a wide geographic scale, in the Palearctic region (Chapter **I)** and on a more local scale, in Southern Finland (Chapter **III**). I also investigated their reproductive isolation using both genetic and behavioural approaches (Chapter **IV**). The aim was to quantify the extent of genetic differentiation and gene flow between *M. rubra* and its parasite, clarify if they are reproductively isolated, and to find out whether they represent an example of sympatric speciation through social parasitism.

The main study questions of each Chapter are summarised in Table 1.

Table 1. The main study questions, results and their implications of the chapters of this thesis.

	Main study questions	Main results and their implications
I	Have Pleistocene climatic cycles affected the populations of *Myrmica rubra*? Where did *M. rubra* find refugia during the last glaciation and how did the species recolonize its present areas of distribution?	Yes. *Myrmica rubra* survived the last glaciation in several refugia located over an extensive area from the west to east. It recolonized its present areas of distribution along several recolonization routes from the south-west and south-east, and probably also from the east.
	Do *M. rubra* and its parasite differ in their mitochondrial DNA?	Locally, the parasite and host usually belonged to the same haplogroup but differed in their haplotype frequencies. The local host and parasite were more closely related to each other than to hosts or parasites in other populations, respectively. Thus their mitochondrial lineages have not diverged.
II	Do *M. rubra* and *M. ruginodis* have different phylogeographic structures and refugia owing to differences in their life-history traits and cold tolerance?	No. Both species had similar west-east phylogeographic structures. Circumstantial evidence, however, supported the hypothesis of additional, more northern refugia of the cold-tolerant *M. ruginodis*.
III	Do *M. rubra* and its parasite differ in their nuclear DNA?	Yes. Microsatellite data showed substantial genetic differentiation between *M. rubra* and its parasite with little hybridisation. The local hosts and parasites were more closely related to the hosts and parasites in other populations than to each other, respectively, which implies speciation of the parasite.
	Does the parasite produce workers?	Yes. A portion of workers was produced by the parasite, which suggests that it is in an evolutionary time scale still a young parasite.
IV	Do *M. rubra* and its parasite mate assortatively?	Yes. Host males mated more often with host than parasite females. Assortative mating can create a behavioural mating isolation, which is a prerequisite for sympatric speciation.
	Are host males produced in the parasitized nest of *M. rubra*?	Yes. 22% of the males were produced by the host queens and workers. This may cause hybridisation of the parasite and host.

3. MATERIALS AND METHODS

3.1. Data

For Chapters **I** and **II** we collected and received from our colleagues nest samples of *Myrmica rubra* and *M. ruginodis* over a wide area in the Palearctic (Fig. 2). We also used DNA sequences from GenBank. Consequently our data consisted of 419 female specimens of *M. rubra* (48 microgynes, 169 macrogynes and 202 workers,) from 95 localities and 202 female specimens of *M. ruginodis* (48 queens and 154 workers) from 53 localities (Fig. 2). In addition, for phylogenetic analyses we used two specimens of *M. kotokui* and Turkish *Myrmica* sp., and one specimen of *M. rugulosa* (for GenBank numbers, see **I** and **II**). For Chapter **III**, we collected 80 parasitized nest samples of *M. rubra* from 11 locations in Southern Finland (Fig. 2), including 511 microgynes, 136 macrogynes and 500 workers. For the genetic part of Chapter **IV**, we collected 21 nest samples of *M. rubra* from five locations in Southern Finland (Fig. 2) and our data consisted of 208 males, 120 microgynes, 52 macrogynes and 116 workers. The female individuals (microgynes, macrogynes and workers) were also used in Chapter **III**. For the behavioural part of **IV**, we collected 19 nest samples of *M. rubra*, nests where the microgynous parasite was found (n = 8) and non-parasitized nests (n = 11) from two populations in Southern Finland (Fig. 2). In mating experiments, we used 259 alate (winged, unmated) microgynes and 259 alate macrogynes, 151 host males and 108 parasite males.

3.2. Molecular markers

Different DNA types and molecular markers can provide answers to different questions on different evolutionary time scales, which is why several DNA types and molecular markers should be used when studying genetic differentiation and speciation of populations (Avise, 2004). We used both mitochondrial DNA (mtDNA; **I** and **II**) and nuclear DNA microsatellites (nDNA; **III** and **IV**). These two DNA types usually give congruent results, though sometimes their signals differ (Zink & Barrowclough, 2008; Toews & Brelsford, 2012). MtDNA is inherited mostly maternally, and thus it reveals only the matrilinear history of the species, whereas nDNA is biparental and passes genetic information from both parents (Avise, 2004).

MtDNA has many features that make it very useful at population-level studies within species or among closely related species. MtDNA is usually neutral and lacks recombination due to its maternal inheritance (Avise, 2000, 2009; but see Dowling *et al.*, 2008). It has a high mutation rate and genetic variability, and low effective population size and thus rapid coalescence time (Avise, 2000, 2009). For these reasons mtDNA is an excellent molecular tool in phylogeographic studies (Avise, 2009). Lately, however, the use of mtDNA in phylogeographic studies has received criticism, since it may sometimes experience recombination or paternal leakage, which can obscure the results (Dowling *et al.*, 2008; White *et al.*, 2008; Galtier *et al.*, 2009; Toews & Brelsford, 2012). For the phylogeographic studies (**I** and **II**), we used two partial mitochondrial gene fragments, cytochrome c oxidase one (COI) and cytochrome b (Cyt b).

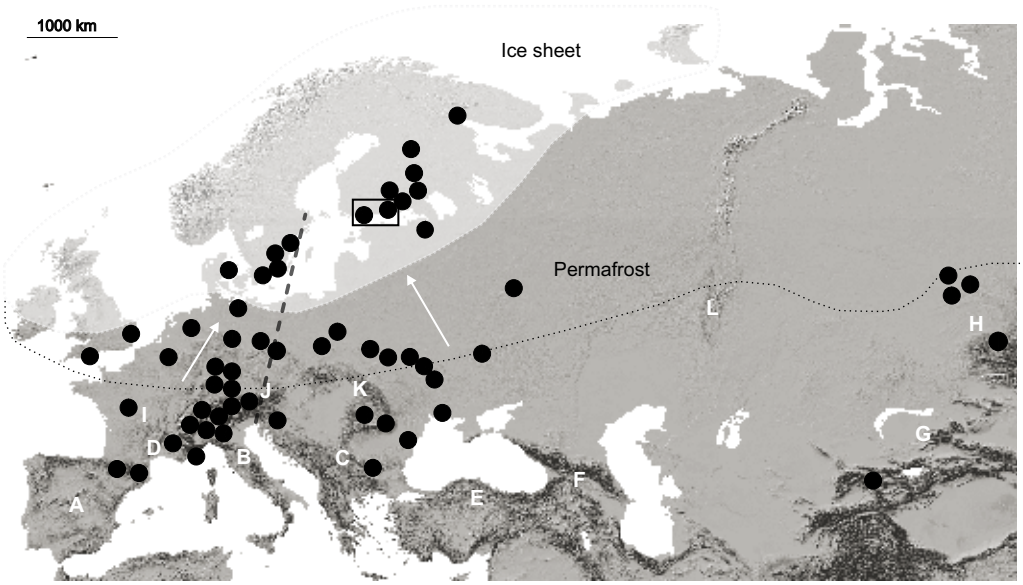
We studied nDNA of *M. rubra* using microsatellites (**III** and **IV**) that are short tandem sequence repeats with varying numbers in a particular location of a chromosome (Avise, 2004). Mutation rate of microsatellites is high and thus, they are informative when studying recent history of populations. We used 11 microsatellite primers.

For details of the laboratory protocols of DNA extraction, mtDNA sequencing and microsatellite genotyping, see the Materials and Methods sections of Chapters **I, III** and **IV**.

3.3. Defining potential refugia

To locate suitable refugia of *M. rubra* and *M. ruginodis*, we combined the knowledge of their biology, the climate (Ganguly *et al.*, 2009) and habitats (Kottek *et al.*, 2006; Allen *et al.*, 2010) of their present areas of occurrence, to the reconstructed information on climate and permafrost (Renssen & Vandenberghe, 2003; Hubberten *et al.*, 2004; Saito *et al.*, 2009; Strandberg *et al.*, 2011) and habitats (Tarasov *et al.*, 2000; Willis & van Andel, 2004; Allen *et al.*, 2010) during the LGM (see details in Introductions of **I** and **II**). We located the potential refugia of the species to the southern European peninsulas (A–C) and to more northern and eastern regions (D–L, Fig. 2). We expected that *M. ruginodis* may have survived in more northern refugia than *M. rubra* (I–L, Fig. 2).

(a) *Myrmica rubra*



(b) *Myrmica ruginodis*

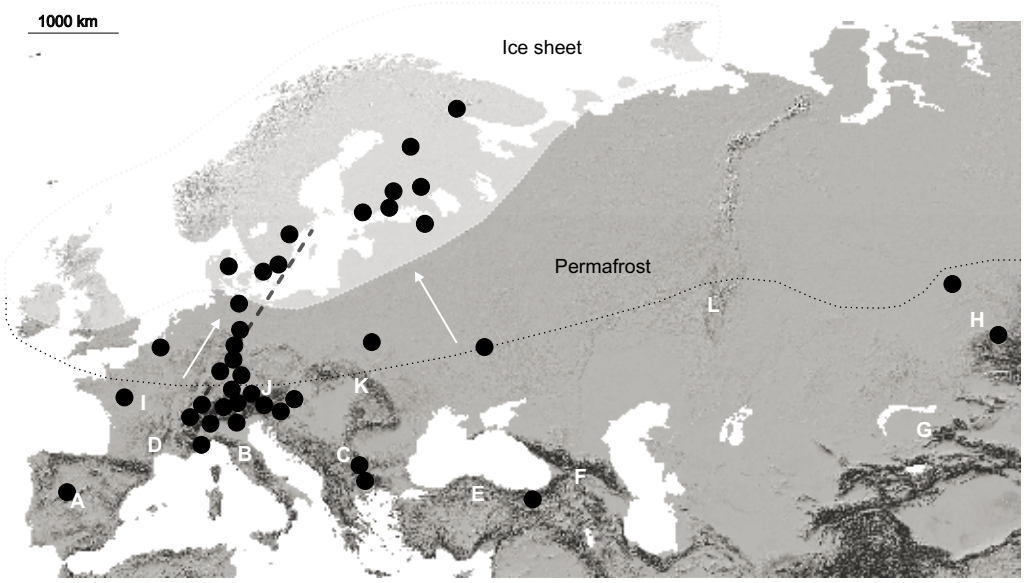


Fig. 2. Sampling locations of (a) *Myrmica rubra* and (b) *M. ruginodis*. A single dot represents one location, except for neighbour-population sets (see details in Chapters **I** and **II**). In (a) a box in Southern Finland represents the sampling area of Chapters **III** and **IV** (see detailed map in **III**). White area is the approximate limit of the ice-sheet (Svendsen *et al.*, 2004), dotted line is the permafrost during the last glacial maximum (Renssen & Vandenberghe, 2003; Saito *et al.*, 2009) and dashed line in Central Europe represents west–east division of the haplogroups. Approximate locations of potential refugia of *M. rubra* and *M. ruginodis* are shown in white letters: A = the Iberian Peninsula, B = the Apennine Peninsula, C = the Balkans–Southern Carpathians, D = southern France, E = Turkey, F = the Caspian–Caucasus region, G = Middle Asia, H = Siberia, I = central France, J = southern Central Europe, K = the Northern Carpathians and L = the Ural Mountains. White arrows indicate directions of postglacial recolonization of *M. rubra* and *M. ruginodis*.

3.4. Phylogenetic analyses

In Chapters **I** and **II** we investigated the history of genetic lineages of populations of *M. rubra* and *M. ruginodis* to clarify their population history and phylogeography. From the mitochondrial sequences, we defined the genetic lineages, the haplotypes. Individuals that have the same haplotype are identical with the studied sequences. To uncover the genetic relationships of haplotypes, we reconstructed a haplotype network with the statistical parsimony approach for each species (Templeton *et al.*, 1992). Haplotype networks use information on inferred mutational steps between the haplotypes and closely related haplotypes can be grouped together into haplogroups (Templeton *et al.*, 1987; Panchal & Beaumont, 2007). Unlike phylogenetic trees, haplotype networks can display multifurcations and place haplotypes at nodes, which can be used to infer ancestor-descendant relationships of the haplotypes (Mardulyn, 2012).

We resolved the genetic relationships of *M. rubra* and *M. ruginodis* (**II**) by reconstructing a haplotype phylogeny that included also sequences of *M. kotokui*, Turkish *Myrmica* sp. and as an outgroup *M. rugulosa*. We applied Bayesian phylogenetic inference (Ronquist & Huelsenbeck, 2003) of the concatenated dataset of COI and Cyt b. To assess if the populations of *M. rubra* and *M. ruginodis* have experienced recent expansions, we examined the number of alleles in haplogroups (Fu, 1997) and performed mismatch distribution analyses (Rogers & Harpending, 1992) in Chapters **I** and **II**. Based on the mismatch distribution analysis, we estimated the mutation rate of the sequences and then estimated divergence times of the haplogroups by using the net distance approach (Tamura *et al.*, 2007).

3.5. Genetic diversity and differentiation of populations

Genetic diversity is an informative measurement in phylogeographic studies, since areas with high genetic diversity are usually either refugia or secondary contact zones of genetic lineages (Hewitt, 1996). To locate the glacial refugia and postglacial recolonization routes of *M. rubra* and *M. ruginodis* in Chapters **I** and **II**, we studied geographic distribution of mitochondrial genetic diversity. We illustrated the results of the distribution of genetic diversity with a genetic landscape showing the areal genetic diversity (Miller *et al.*, 2006) and with maps showing haplogroups of populations. In Chapter **III,** we compared genetic diversity of populations and different castes of *M. rubra* to test, if microgynes were genetically less diverse than macrogynes.

To study the speciation of *M. rubra* and its parasite, we measured genetic differentiation between micro- and macrogynes with different hierarchical approaches in Chapters **I** and **III**. We tested what portion the different hierarchical levels explained of the total variation. We tried alternative orders of the hierarchical levels to find out whether the highest level in the most trustworthy order was the queen morph or population.

We examined genetic similarity of the groups within populations in Chapters **III** and **IV**, by performing a principal component analysis (PCA) based on the covariance matrix and the allele frequencies of nest samples of each female caste. In addition, in Chapter **III** we estimated the number of genetic clusters within each population and hybridisation between the clusters by performing mixture and admixture analyses of the populations with Bayesian clustering (Corander & Marttinen, 2006; Corander & Tang, 2007; Corander *et al.*, 2008) by including all different castes as separate units.

3.6. Reproduction of *Myrmica rubra* and its social parasite

To clarify the reproduction of queen morphs, we studied genetic identity of *M. rubra* females (**III**) and males (**IV**) in the parasitized nests. In Chapter **III,** we identified which queen group (macrogynes or microgynes) had produced workers in the population based on the Bayesian clustering (Corander & Marttinen, 2006; Corander & Tang, 2007; Corander *et al.*, 2008). In Chapter **IV**, to find out the genetic origin of males we used the maximum likelihood approach based on allele frequencies of males, macrogynes, microgynes and workers (Hastings *et al.*, 1998). Last, we studied the mating preferences of *M. rubra* and its parasite in laboratory conditions by allowing the parasite and host males to mate with either the parasite or host females (**IV**).

4. RESULTS AND DISCUSSION

4.1. Phylogeography of *Myrmica rubra* and *M. ruginodis*

4.1.1. Phylogeographic structures

On the contrary to our expectations, differences in the life-histories of *M. ruginod*is and *M. rubra* have not resulted in different phylogeographic structures. *Myrmica ruginodis* that is characterized by more efficient dispersal did not have less differentiated populations and a weaker phylogeographic structure than *M. rubra*. Instead, the populations of *M. ruginodis* were genetically only slightly more homogeneous than those of *M. rubra* and the species had similar phylogeographic structures (Fig. 1 in **II**). Thus, the differences in the population structures of the two species are only present in nuclear microsatellites (Seppä & Pamilo, 1995; Nash *et al*., 2008; Vepsäläinen *et al*., 2009) but not in mitochondrial DNA.

Most haplogroups of both species were widely distributed, but western and eastern populations of both species belonged mostly to different haplogroups (dashed line in Fig. 2. represents the west–east division). This kind of west–east phylogeographic division has been detected in many temperate taxa (Taberlet *et al*., 1998), including several ant species (Goropashnaya *et al*., 2004; Pusch *et al*., 2006; Schlick-Steiner *et al*., 2007), and it can be used to localise refugia and postglacial recolonization routes of the species.

4.1.2. Refugia

Comparative phylogeographic studies have shown that most European temperate taxa survived the last glaciation mainly in southern Europe—in the Iberian, Apennine and Balkan peninsulas (Taberlet *et al*., 1998; Hewitt, 1999; Schmitt, 2007; Stewart *et al*., 2010), though indications of more eastern (Grichuk, 1992; Bilton *et al*., 1998; Polyakov *et al*., 2001; Goropashnaya *et al*., 2004, 2007) and northern refugia (Pfenninger & Posada, 2002; Willis & van Andel, 2004; Sommer & Nadachowski, 2006; Ursenbacher *et al*., 2006; Stewart *et al*., 2010) have been discovered for several taxa. Lately, the importance of these southern European refugia as main sources of postglacial recolonization for temperate species has been questioned (Weiss & Ferrand, 2007; Stewart *et al*., 2010) and to better understand phylogeographic patterns in Europe—studies on many additional taxa are needed (Schmitt, 2007; Avise, 2009; Hickerson *et al*., 2010; Stewart *et al*., 2010).

Based on genetic variation and differentiation of populations, distribution of the haplogroups and haplotypes that were either local or frequent and central in a haplogroup, both *Myrmica rubra* and *M. ruginodis* have survived the LGM in multiple refugia in Southern Europe, but also in more eastern and northern regions (**I** and **II**). The results implied that both species had refugia in the Mediterranean peninsulas (A–C, Fig. 2). In addition *M. rubra* likely had a refugium also in Southern Siberia or adjacent areas (H, G). This area may have also been a refugium for *M. ruginodis* but scarce sampling there leaves this very speculative. *Myrmica ruginodis* had a refugium also in Turkey (E) or near the Caspian–Caucasus region (F).

We expected that the cold-tolerant *M. ruginodis* would have survived also in the northern refugia (I–L, Fig. 2, **II**). The distribution of a western haplogroup of *M. ruginodis* points to a refugium in central France (I), or alternatively, in southern Central Europe (J) where also *M. rubra* may have survived. From the other northern refugia, the northern Carpathians (K) and the Ural Mountains (L), we have no data. Both have likely been refugia for several taxa (Bilton *et al*., 1998; Polyakov *et al*., 2001; Goropashnaya *et al*., 2004, 2007; Provan & Bennett, 2008; Kuzmina, 2009; Mardulyn *et al*., 2009; Wójcik *et al*., 2010). The habitat in the northern Carpathians (K) was probably suitable for *M. ruginodis* during the LGM (Kuneš *et al*., 2008), but based on the LGM vegetation maps (Frenzel, 1992; Grichuk, 1992; Tarasov *et al*., 2000; Allen *et al*., 2010) there is incongruent evidence whether suitable habitat for *M. ruginodis* (Radchenko & Elmes, 2010) was found also in the Ural Mountains (L) during the LGM.

4.1.3. Postglacial recolonization

Haplogroups of both *M. rubra* and *M. ruginodis* showed signs of recent expansion that according to our estimates occurred during or after the last glaciation (**I** and **II**). The west–east phylogeographic structures of both species suggest that populations colonized northern areas using both western and eastern recolonization routes. Evidently, populations of *M. ruginodis* in Iberia (A) and in Turkey (E, or Caspian–Caucasus refugium F) did not expand their distribution after the LGM. Also the local haplotypes in Italy imply that the Apennine Peninsula (B) was not a source of postglacial recolonization.

Of the two western haplogroups of *M. rubra*, one points to Iberian Peninsula (A) as a source of recolonization. The refugium of the other western haplogroup of *M. rubra* and the western haplogroups of *M. ruginodis* is, however, difficult to localise. These haplogroups may have originated in central France (I), or that of *M. ruginodis* also west of the Alps.

Owing to scarce sampling and the wide distribution of the eastern haplogroups, the eastern recolonization routes are difficult to localise. The eastern recolonization may have started anywhere between the Apennine Peninsula (B) and southern Siberia (H) with the Balkans–Southern Carpathians (C) in between. Since both species—especially *M. ruginodis*—are cold-tolerant (Kipyatkov, 2001; Czechowski *et al.*, 2012) and have probably survived in many regions (see 3.3. above), these eastern refugia may have been interconnected or they may have formed one large eastern refugium.

4.2. Genetic differentiation and reproductive isolation of *Myrmica rubra* and its social parasite

4.2.1. Mitochondrial DNA sequences versus nuclear DNA microsatellites

The haplotype network (Fig. 3 in **I**) based on mtDNA showed little evidence for lineage sorting of the parasite from its host. The parasite and host from the same nest usually differed in their haplotypes, but the parasite was mainly more closely related to its local host than to parasites from other populations, respectively (**I**). When mtDNA was studied more locally in the populations of Southern Finland (**III**), the levels of population and queen morph were almost equally important.

In contrast to mtDNA, in nDNA microsatellites the queen morphs clustered into separate genetic groups with little hybridization (Fig. 4 in **III**) and explained a higher proportion of the genetic variation than did the populations. The local hosts and parasites were more closely related to the hosts and parasites in other populations than to each other, respectively. The populations of the parasite and host showed high genetic differentiation (F_{ST} = 0.375). This is close to the amount of genetic differentiation found between the two distinct species, the social parasite *Ectatomma parasiticum* and its host *E. tuberculatum* (Hora *et al.*, 2005, 2009).

The microgynes of *M. rubra* were extremely homozygous, which is probably a result from high inbreeding (Keller & Waller, 2002) and microgynes mating with their relatives near their natal nests. The alternative option—parthenogenetic reproduction observed in several ant species (Wenseleers & Van Oystaeyen, 2011)—cannot, however, be ruled out since it may also result to excess of homozygosity.

The genetic data of this thesis shows that the parasite and host are genetically differentiated in their nDNA microsatellites but their mitochondrial lineages have not yet diverged. This supports the synonymization of the parasite and host by Steiner *et al.* (2006) but implies that they are probably in an early stage of speciation. Some of the genetic differentiation may, however, also result from the recurrent bottlenecks and genetic drift of the parasite populations (Vepsäläinen *et al.*, 2009) as well as from their excess of homozygosity. It is difficult to predict, if the speciation process of the parasite continues and results to divergence of mitochondrial lineages. The close relationship of social parasite species and their hosts (see the references in 1.3.) would nevertheless suggest that this kind of situation may lead to origin of new species if mating is assortative.

4.2.2. Assortative mating

In *Myrmica* ants, mating is typically preceded by swarming behaviour. Sexual individuals, alate gynes and males, leave their natal nests and gather in mating swarms in an open area to find mates (Elmes, 1991a and the references within). Brian & Brian (1955) argued that in queen dimorphic species the queen morphs have both temporal and spatial differences in mating. During the mating time, macrogynes fly away from the nest to mate in swarms whereas microgynes mate inside or near their natal nest (Brian & Brian, 1955; Kasugai *et al.*, 1983; Elmes, 1991a; but see Buschinger, 1997). Microgynes of *M. ruginodis* have been found from mating swarms (Elmes, 1991a) but those of *M. rubra* presumably mate inside or near their natal nests (Buschinger, 1997; Seifert, 2007; own ubpublished observations). We have, however, found alate microgynes outside their nests and once macrogynes mating on top of their nests (own unpublished observations), indicating that the possible spatial isolation between the morphs may sometimes be broken.

In **IV** we observed that *M. rubra* and its parasite mated in laboratory conditions. Host males mated

significantly more often with host than parasite females, which indicates positive assortative mating among host individuals. This may act as premating mechanism and prevent hybridization of the parasite and host. Parasite males did not, however, mate significantly more often with parasite females than host females. In the parasitized nests of *M. rubra*, host males—but not females—may be present (**IV**, own unpublished observations). If the parasite mates in or near its natal nest, it is probably more important to prevent hybridization of the host males and parasite females than the host females and parasite males.

4.2.3. Production of different castes in the parasitized nests

In an ant nest, both workers and queens are able to control the reproduction of the other female individuals (Elmes, 1980, 1991b; Hölldobler & Wilson, 1990). Usually one or a few dominant queens inhibit the reproduction of the other queens and workers, and influence the caste determination of their offspring (Keller, 1995). This is referred to as the queen effect (Elmes & Wardlaw, 1983). Due to the queen effect, other queens than the dominant ones produce only workers and workers only trophic eggs. Reproductive control probably occurs through physical and chemical control (Brian, 1954, 1970, 1973; Brian & Carr, 1960; Bourke, 1988; Walin *et al.*, 1998; but see Brian 1969 and Smeeton, 1981).

When an inquiline is present in the nest, sexual offspring is usually produced by the parasite (Buschinger, 1986, 2009). Thus, we expected that the males in the parasitized nest of *M. rubra* are produced by the microgynes. Earlier Elmes (1976) has noted that the microgynes of *M. rubra* can suppress the growth of the macrogynous larvae, which supports this expectation.

The results of Chapter **IV** showed that in most parasitized nests of *M. rubra* (n = 9), all males were produced by parasites, but in total 22% of the males were produced by the host queens (11%) and workers (12%; Figs 5 and 6 in **IV**). The host males were significantly larger than the parasite males but as in the data of Seifert (1993), there was a considerable overlap in their size distributions (Fig. 7 in **IV**). Our results suggest that the host can sometimes produce sexual offspring in the presence of the parasite. If the parasite mates inside or near its natal nest, the presence of the host males may cause hybridization between the parasite and host. Therefore, also other features than size, for example chemical cues, are probably present in the mate recognition of the parasite.

Microgynes of *M. rubra* have produced workers in laboratory conditions (Elmes, 1976), and in the genetic study of Vepsäläinen *et al.* (2009) some workers were likely produced by the microgynes. Pearson & Child (1980), however, argued that microgynes probably produce workers only infrequently in the field. In the Chapter **III,** we studied the hypothesis that the inquiline produces no or few workers (Buschinger, 2009).

We found that a small portion (8%) of workers in the parasitized nests was produced by the microgynes. Also other inquilines have been found to produce workers, but their ability to perform normal colony tasks is often decreased and they may be more fertile than the host workers (Wilson & Brown, 1956; Sumner *et al.*, 2003; Hora *et al.*, 2005). Elmes (1976) reported that also the workers of *M. rubra* microgynes seemed to be less efficient in colony tasks than those of macrogynes.

Only some of the nests had parasite workers and thus, perhaps the parasite produces workers only in certain conditions. The caste determination of *Myrmica* is plastic and dependent, for example, on worker control and nutrition of the larvae (Brian, 1956; Elmes, 1980, 1991b; Elmes & Wardlaw, 1983). If some of the parasite larvae do not receive enough nutrition and care from the workers, instead of developing into gynes they may develop into workers. Nevertheless, the result that the parasite of *M. rubra* produces workers implies that it is in an evolutionary time scale still a young parasite, as the phylogenetic analyses have also shown (Savolainen & Vepsäläinen, 2003; Jansen *et al.*, 2010; Leppänen *et al.*, 2011).

4.2.4. From queen dimorphism to social parasitism and speciation

The size of ant queens may either be induced by the environment or it may have a genetic basis (Heinze & Tsuji, 1995; Heinze & Keller, 2000). The queen morphs often differ in their reproductive strategies (Heinze & Tsuji, 1995). In some species such as *M. rubra* detailed observations have revealed that microgynes are social parasites (Heinze & Tsuji, 1995), whereas sometimes queen dimorphism has been mistakenly treated as social parasitism (Bolton, 1986). One ant genus where both queen size-dimorphism and social parasitism is present is the genus *Myrmica* where an evolutionary sequence may exist from queen dimorphism into

social parasitism and speciation (Brian & Brian, 1955; Elmes, 1978; Pearson, 1981; Seifert, 2010 and the references within). The results in this thesis support this hypothesis.

Several features make the nests of *M. rubra* very vulnerable for social parasitism. The nests are highly polygynous and polycalic (Elmes, 1980; Seppä & Pamilo, 1995) and its workers often favour new queens over the old ones (Elmes & Brian, 1991). In the past, microgynes have changed their reproductive strategy and started to behave parasitically, concentrating mainly on sexual reproduction. Laboratory studies of Pearson (1981) and Elmes (1976) have shown that microgynes differ in their reproduction from the macrogynes, which has likely supported their parasitic lifestyle. First, the microgynes produce more sexual offspring than the macrogynes (Pearson, 1981), which is a clear fitness benefit for them. Second, microgynes can develop into gynes from small larvae that would normally develop into workers (Elmes, 1976). This supports the hypothesis that the small size of the parasites helps them to escape the caste control and instead of developing into workers, they can develop into queens. Third, microgynes have produced new gynes also in the rapid brood—exceptional in the genus *Myrmica*—and thus they do not need to overwinter to finish their metamorphosis like the macrogynes (Elmes, 1976). All the above observations indicate that the microgynes have a clear reproductive benefit that has likely been favoured for. If selection is strong and microgynes mate assortatively—as seems likely—prerequisites for sympatric speciation (Smith, 1966; Gavrilets, 2003) have been reached.

5. CONCLUSIONS

This thesis shows that genetic variation and differentiation of *Myrmica* populations may result both from environmental factors, such as the climatic cycles affecting the distribution of suitable habitats (**I** and **II**), and behavioural factors, such as alternative reproductive tactics (**I**, **III** and **IV**). The Chapters **I** and **II** clarify the Pleistocene refugia and subsequent postglacial recolonization of extensively distributed Palearctic insects, and render information how Pleistocene climatic cycles have affected their populations. The Chapters **I**, **III** and **IV** shed light on genetic differentiation and reproductive isolation of a social parasite and its host, and provide new evidence for possible sympatric speciation.

Differences in life-histories of *M. rubra* and *M. ruginodis* did not have an effect on their phylogeographic structures. Their populations survived the Pleistocene glaciations in several refugia, likely in the traditional Mediterranean refugia but also in more northern and eastern regions (**I** and **II**). Only circumstantial evidence supported that *M. ruginodis* survived in more northern refugia than *M. rubra*. The difficulty of defining the eastern refugia and postglacial recolonization of both species may be a result of several neighbouring refugia, or alternatively, one large eastern refugium. In phylogeographic studies the eastern regions should be sampled in more detail than has usually been done. More detailed sampling in the east may change our view about the main refugia and postglacial recolonization of the Western Palearctic species.

The queen morphs of *M. rubra* showed high genetic differentiation with little hybridization in nDNA microsatellites, suggesting incipient speciation (**III**). Though the parasite and host often differed in mitochondrial haplotypes, their mitochondrial lineages have not yet diverged (**I**). Mating experiments implied that assortative mating probably keeps the parasite and host apart (**IV).** The genetic origin of individuals in the parasitized nests showed that the host queens and workers may sometimes produce males (**IV**) and the parasite occasionally produces workers (**III**). This implies that the microgynous morph is still in evolutionary time scale a young parasite.

In conclusion, the parasite of *M. rubra* is a good candidate for sympatric speciation through intraspecific social parasitism. Future studies should focus on the reproductive isolating mechanisms in the field to investigate whether the parasite and host are spatially or temporally isolated during the mating time. The underlying factor behind the excess of homozygosity of the microgynes is also an important aspect and would clarify the population structure and behaviour of the parasite as well as the amount of genetic differentiation between the parasite and host. Last, to widen our understanding about the possible evolutionary sequence from queen dimorphism to social parasitism and speciation in *Myrmica*, we should also study queens of the other species of the genus, especially *M. ruginodis.*

Jonna Kulmuni, Tuomas Leinonen, Riitta Savolainen, Perttu Seppä, and Laura Kvist gave valuable comments on this summary.

6. ACKNOWLEDGEMENTS

This journey has been long and I have not done it alone. Many people have helped and supported me over the years, for which I am very thankful.

First of all, I would like to thank Riitta and Kari for hiring me and giving me the opportunity to make this PhD even though I had so little experience in genetics. You have taught me many things about ants, science and life over the past years, thank you for all your advice. I am very grateful to Riitta since I could always come to your office to ask advice and you always helped me even with the last minute questions. Kari, thank you for persistently teaching me how to write. You did not give me straight answers and forced me to think, however, having always new ideas if I had none. Sometimes working has been hard for all of us and I am now very proud of us that we have succeeded and that my thesis is now ready.

Next I owe big thanks to Perttu, who has helped me enormously. You had always time to teach me population genetics and discuss for hours about weird life of the microgynes. Whatever my problem was, you helped me and when I only saw a dead end, you gave me suggestions how to continue.

I would like to thank Dr. Laura Kvist and Prof. Jes Pedersen for pre-examining and commenting my thesis. I am very grateful to all the myrmecologists who provided us samples and commented my work. This work has been inspired by Dr. Graham Elmes who has done numerous excellent studies on *Myrmica rubra*. I was honored to meet Graham in *Myrmica* minisymposium in Tvärminne and I appreciated our discussion and his comments concerning my work.

I owe my gratitude to my excellent thesis advisory committee: Jukka, Lotta and Perttu. I decided to establish this group to help me finish my PhD. Thank you for your comments and support and looking after my schedule.

My dear PhD brother was Gunther. Thank you for your support, help and friendship. I know that if I had not finished my PhD you would have come and kicked my ass. I would like to thank Hannele, who brought sunshine to the lab. You did great and it was a pleasure to work with you. Thank you for your friendship as well.

Anni, you have also been the great support during these years. You have not only done your official duty, but you really cared for us PhD students and asked how we were. Thanks to Jussi for taking care of all the paper work so well. I am very thankful for Leena Liikanen who took care of my money and advised me with all the bureaucracy.

I am especially thankful to the 11.15 lunch group and its former members for your support, laugh and friendship: Vilppu, Tuomas, Hannu, Ed, Daniel, Diego, Nolwen, Riikka, Aksu, Kimi, Göran and Jaana. I really enjoyed our lunch breaks and all the great discussions that we had. I will miss these happy breaks in Gardenia. Vilppu, you have seen the good and bad until the very end. It was great to have such a good friend close by during this trip and it is great to finish this together. Thank you for all the support. Big thanks also to Tea for all your help, support and friendship. I could always share my success and sorrows with you.

I would also like to thank all the people at the department for the nice atmosphere. I am thankful to Joona for the help with the maps. Big thanks to Heini K. for all your advice with the practical things before the defence. Jonna, I was very happy that you moved to Helsinki. Thank you for your support and having such a positive attitude. We had many great discussions. Thanks for nice discussions also to Emma, Helena J, Helena W and Misha. Itsuro and Raisa, thank you so much for your help with the phylogeographic methods.

I could not have done this without the MES lab. I am thankful for all the lab people, especially Heini A., Heidi, Kalle, Minttu, Marika, Kirsi, Lena, Leena, Toshka and Suvi for your help. Marika, you always helped me whatever my question was which was just great.

I had excellent roommates over the years: Diego, Gunther, Harri, Maaike, Natalia, Nolwen, Patte and Philip. Thank you for the nice atmosphere, help and support–and being so quiet.

Thank you very much for my dear friends for the support and happy moments! Hopefully I will now see you more often.

I would like to thank my family for all the support over the years. Mum and dad, I can always count on you. Thank you Mari and Mainostoimisto Muikku for making this thesis readable in such a short time. And sorry for the equation and boring tables. Rakkaalle Aili-mummille suuri kiitos kannustuksesta kaikkien näiden vuosien varrella. Thank you little Amelie for taking my thoughts away from science and patiently waiting me in the window when I had to work late.

I am also grateful to Mari and Petteri for borrowing Amelie for me.

Jarkko, words are not enough to thank you for your endless support. Even in the deepest moments you have kept me standing and made me believe in myself. You have also helped me with my field work in Bulgaria and Finland. Thank you for your patience and understanding, and I am sorry for all the holidays I skipped.

Last, I would like to thank all the foundations that financed my work and my referees for believing in me.

This thesis has been funded by the Academy of Finland (project no. 210523 to RS and a travel grant to JL), the Alfred Kordelin Foundation, the Ella and Georg Ehrnrooth Foundation, the Finnish Cultural Foundation, Helsingin Hyönteistieteellinen Yhdistys, the Kuopio Naturalists' Society, the Oskar Öflund Foundation, the Otto A. Malm Foundation, Societas pro Fauna et Flora Fennica and the University of Helsinki.

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